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**Evaluation of Antiviral Agents in Two Mouse
Models of RNA virus infections**

by

John Hyrum McClatchy
under the direction of Dr. E. Bart Tarbet, faculty mentor

A thesis submitted in partial fulfillment of the
requirements for Departmental Honors for the degree

of

BACHELOR OF SCIENCE

in

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Abstract:

The first model we are evaluating is human influenza virus in mice. Influenza virus is a negative sense RNA virus, which the CDC estimates causes between 9.2 million and 35.6 million illnesses each year in the United States.^[1] In the current influenza season, H3N2 viruses have been the predominant subclade of influenza A virus in circulation.^[2] Development of a mouse model for influenza H3N2 virus infection has been difficult. In these studies, we endeavored to mouse-adapt influenza A/Hong Kong/4801/2014 H3N2 virus by serial passaging in AG129 mice. This virus strain was chosen as it overlaps in serotype with the current influenza H3N2 virus in circulation.^[2] Initial passaging was unsuccessful, with no detectable increase in virus titer. However, when we used Mannan, an innate immune suppressant, as an aid in adapting the virus to mice, we observed an increase in virus titers in the lung between passage 1 to passage 3.

The second model we are evaluating is mouse Norovirus as a model for Human Norovirus infections. Human Norovirus is a positive sense RNA virus responsible for approximately 685 million cases every year across the world.^[3] Therefore identification of antiviral agents for treatment of human norovirus is a priority. We screened potential antiviral compounds *in vitro* utilizing a mouse macrophage cell line. rupintrivir, favipiravir, carbodine, and anicomycin were all observed to have antiviral effects *in vitro*. *In vivo* screening of carbodine will be completed in the mouse model as there is no human norovirus model *in vivo*.

Acknowledgments:

First and foremost, I would like to thank Dr. Tarbet for the effort he has put in on my behalf, as well as the patience he's chosen while mentoring me. He has taken the time to not only explain not only logistics of the specific project I am working on, but also to explain the dynamics of the field of research as a whole and how to work within it.

I would also like to thank Chris Peterson, for the time he spent with me discussing possible ways to help various viruses replicate in mice, and for times we spent emailing back and forth potential compounds for testing.

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1 Introduction:

1.1 Influenza H3N2

Influenza viruses are negative sense RNA viruses from the family Orthomyxoviridae. Seven genres exist within Orthomyxoviridae, three of which cause infection in humans; influenza type A, B, and C. Of these three genres, influenza type A and B are the most prevalent in human disease. Both viruses have segmented genomes consisting of eight separate segments, whereas influenza C has seven separate segments. ^[4] Worldwide annual epidemics of influenza virus cause on average 3 to 5 million severe cases of illness, with an estimated 290,000 – 650,000 respiratory deaths. ^[5] In addition to the loss of human life influenza virus also represents an economic burden. In the United States alone influenza virus infection is estimated to cause annual net economic loss of \$47.2 – \$149.5 billion dollars. ^[6]

In our current influenza season, type A influenza has been the predominant form of influenza virus in circulation. ^[2] The type A influenza viruses are subdivided based on which combination of two glycoproteins, hemagglutinin and neuraminidase, they possess. Neuraminidase is found in 11 different subtypes, numbered N1 – N11, all of which catalyze the cleavage of sialic acid, aiding in viral release. ^[7] Hemagglutinin is found in 18 different subtypes, numbered H1 – H18, all of which are involved in initial binding of the virus to sialic acids. ^[7] Of these different subtypes, influenza A subtypes H1N1 and H3N2 make up the vast majority of influenza A infections within humans. ^[2] In this current influenza season, from October 2017 to April 15th, 2018 there have been 36,858 specimens of influenza A and 50,664 positive samples of influenza tested by public health laboratories across the United States. Of the 36,858 samples of influenza A, H3N2 has been the causative agent 84.3% of them. Furthermore, of the 50,664

samples of influenza A and B tested this season, influenza H3N2 was the causative agent in 61.2% of them. ^[2]

Despite the prevalence of influenza H3N2, development of a mouse model for the influenza H3N2 viruses currently in circulation has proven difficult. Given this, there is demand for a mouse model of influenza H3N2 for the evaluation of potential therapeutics against current H3N2 strains. The CDC has demonstrated that 96.9% of the 509 current influenza H3N2 viruses tested were inhibited by ferret antisera generated against A/Michigan/15/2014 (3C.2a) and a cell-propagated A/Hong Kong/4801/2014-like virus. ^[2] This suggests that either strain is likely a suitable target for the development of a mouse model.

1.2 Human and Murine Norovirus

Human Norovirus and murine norovirus, both lie within a genus of the family Caliciviridae, and are positive sense, single stranded RNA viruses. Human norovirus is responsible for approximately 685 million infections every year across the world and is the leading cause of acute gastroenteritis. ^[3] Lethality from norovirus is normally restricted to children, and it is estimated that the virus kills 50,000 children yearly, predominately in developing countries. ^[3] Additionally it is estimated that human norovirus infection causes a global economic loss between \$44.4 – \$83.4 billion dollars. ^[8]

Given the cost to human health and the economic loss resultant from human norovirus infection, the discovery of therapeutics for treatment of the virus is a priority. Despite this, therapeutic development has historically been slowed by a lack of cell cultures capable of supporting human norovirus replication. Instead of in vitro replication to supply stocks of human norovirus, these stocks have been harvested from the stools of hospital volunteers. ^[9] In addition

to difficulties in vitro, there has been no successful development of a reliable, lethal small animal model for therapeutic evaluation. [9]

Given the difficulties with working with human norovirus, a reasonable alternative which has been previously proposed is the evaluation of potential therapeutics in a lethal murine norovirus AG-129 mouse model. Murine and human noroviruses are genetically similar both containing ~7.5 kb genome. Human norovirus has three open reading frames (ORFs) and murine norovirus possesses 4. The first three ORFs encode for equal number of proteins with analogous functions between the two viruses. [10] ORF4, unique to murine norovirus, is encoded within an alternative reading frame of within ORF2. It encodes virulence factor 1, a mitochondrial localized novel innate immune regulator, which represents the major genomic difference between the two viruses. [11] While differences exist, the amount of similarities combined with the ability of murine norovirus to lethally replicate within the RAW 264.7 cell line as well as AG-129 mice, in a manner reversible by effective antiviral compounds make murine norovirus a convenient possible alternative model. [12]

2 Materials and Methods:

2.1 Animals

This work was done in the Association for Assessment and Accreditation of Laboratory Animal Care International-accredited, biosafety level 2 laboratory at Utah State University. Male and female AG129 mice were used and were bred in-house in sterilized cages and maintained in a 12/12 light cycle. Animals were euthanized by cervical dislocation by a trained technician. An immune compromised AG-129 strain of mice was chosen as previous attempts to adapt H3N2 in immune competent strains, such as the BALB/c strain of mice, had proven unsuccessful.

2.2 Initial Virus Stock Influenza H3N2

The viral strain utilized was Influenza A Hong Kong/4801/2014 (H3N2) virus. Prior to passaging in mice, this virus was passaged twice in a Madin-Darby Canine Kidney (MDCK) cell line to generate sufficient stock for inoculation of mice.

2.3 Initial 5 Passages of Influenza H3N2

Mice ages 5 to 8 weeks old were inoculated intranasally (i.n.) with 90 μ l of a 1:1 solution of virus stock and a mannan MEM solution. Prior to inoculation, mice were sedated with Ketamine/Xylazine mixture. Virus harvested from the prior passage was utilized as the stock for infection of the next passage. Inoculation of passage one utilized the initial virus stock, grown in MDCK cells. On day one and day two the mice were given an intranasal rinse of sterile saline at a volume of 90 μ l. Three days post inoculation, mice were euthanized by cervical dislocation for necropsy and harvest of the lungs. Lungs were homogenized in 1 ml of MEM with 50 μ g/ml of gentamycin, for inhibition of bacterial growth in media. Homogenate was divided into two equal aliquots. One for storage, the other aliquot was pooled with those from other mice in the passage. This pooled homogenate was utilized to infect the subsequent passage. Passage one was inoculated with a 50% cell culture infectious dose per ml (CCID₅₀) of $10^{4.7}$. Passage two was inoculated with a CCID₅₀ of $10^{3.97}$. Passage three was inoculated with a CCID₅₀ of $10^{5.36}$. Passage four was inoculated with a CCID₅₀ of $10^{5.91}$. Passage five was inoculated with a CCID₅₀ of $10^{5.53}$. All CCID₅₀ values have been adjusted for the $\frac{1}{2}$ reduction from dilution by mannan MEM solution.

2.4 Evaluation of Disease Markers in MP4

Mice ages 5 to 8 weeks old were inoculated i.n. with a 90 ul, 1:1 solution of MP4 (CCID50 of $10^{5.53}$, already adjusted for dilution with mannan) and a mannan MEM solution. Prior to inoculation, mice were sedated with Ketamine/Xylazine mixture. Mice were sedated with the same Ketamine/Xylazine mixture on day one and two for administration of an intranasal rinse of sterile saline. Mortality was checked, and mice were weighed daily until euthanasia by cervical dislocation on day 20.

2.5 Influenza H3N2 Passage with no Mannan and Rinses

Mice ages 5 to 8 weeks old were divided into three groups, labelled group 1, 2 and 3. All mice were sedated before viral inoculation. Group 1 was inoculated intranasally with MP3 (at a CCID50 of $10^{6.2}$) and group 2 was inoculated intranasally with MP5 (at a CCID50 of $10^{6.0}$). Group 3 was inoculated with a MP5 (at a CCID50 of $10^{5.8}$, already adjusted for dilution with mannan). Both group 1 and two were not given mannan. All three groups were not given intranasal rinses on day one or two. Mice were euthanized, and the lungs removed during necropsy on the third day post infection. In subsequent passaging, all procedures remained the same, except mice were inoculated with a homogenate from their group's previous passage.

2.6 Intranasal Rinses

Initial attempts at passaging of the influenza H3N2 virus in AG-129 mice were unsuccessful. In response to this, intranasal rinses were given on day 1 and 2 as they are known to increase viral titer, and the likelihood of mortality.^[13] This effect is possibly mediated by rinsing the virus deeper into the lungs, exposing it to new cell populations.

2.7 Mannan

Mannan is a polysaccharide preparation from *Saccharomyces cerevisiae*. It has been shown previously to increase the ability of influenza A to survive by inhibiting a host defense collectins, namely mannan-binding lectin, by competing for binding sites with the virus.^[14] Mannan-binding lectin is a pathogen recognition receptor which can inhibit infection by complement activation (through the lectin pathway), opsonization, and direct viral neutralization.^[15] The optimal range for inhibition of collectins for amplification of influenza virus growth has been demonstrated previously in other strains of influenza virus to be .5 mg/mouse, therefore this is the dose we utilized.^[16]

2.8 Cells and Media Influenza H3N2

Madin-Darby canine kidney Atlanta (MDCK-ATL) cells, were obtained from the International Reagent Resource (IRR) which is managed under contract by the American Type Culture Collection (Manassas, VA) and maintained at 37 °C and 5 % CO₂ in antibiotic-free cell culture medium (minimum essential medium with Earle's balanced salts and L-glutamine, MEM/EBSS) supplemented with 5 % fetal bovine serum (both from HyClone, Fisher Scientific, Logan, UT). The test medium consisted of MEM/EBSS supplemented with trypsin (10 U/ml), EDTA (1 µg/ml) and gentamicin (50 µg/ml).

2.9 Cells and Media Murine Norovirus

RAW 264.7 murine macrophage cell line were obtained from the American Type Culture Collection (Manassas, VA) and maintained at 37 °C and 5 % CO₂ in antibiotic-free cell culture medium (minimum essential medium with Earle's balanced salts and L-glutamine, MEM/EBSS)

supplemented with 10 % fetal bovine serum (both from HyClone, Fisher Scientific, Logan, UT). The test medium consisted of MEM/EBSS supplemented with 2% fetal bovine serum and gentamicin (50 µg/ml).

2.10 Quantification of Influenza H3N2

The estimated number of virions of influenza H3N2 in all mouse studies was quantified by dilution to extinction down 8 wells of a 96 well plate. This plate was seeded with 4×10^4 MDCK-ALT cells the day prior. Results were read visually at three days post infection. Visual reads of cell death were confirmed four days post infection by the neutral red uptake method. Plates were treated for 2 h at 37 °C with neutral red (0.011 %) to quantify CPE. Excess dye was rinsed from cells with phosphate-buffered saline. The absorbed dye was eluted by addition of 0.1 ml of 50 % Sorensen's citrate buffer/50 % ethanol to each well. Optical density (OD) measurements were taken using a computerized microplate reader (SPECTRAmax® Plus384, Molecular Devices Corporation, Sunnyvale, CA) with absorbance measurements at 560 nm. The CCID50 was determined using the Reed-Muench method.

2.11 Murine Norovirus

The viral strain used was Murine Norovirus (MNV-1.CW1). Prior to passaging in mice, this virus was passaged once in a RAW 264.7 cell line to generate sufficient stock for inoculation of mice.

2.12 Antiviral Compounds Against Murine Norovirus

The compounds tested were; rupintrivir, carbodine, favipiravir, anisomycin, pirodavis, guanidine, cyanovirin, azacytadine, 1-Beta-D-arabinofuranisyl and nitazoxanide. azacytadine was obtained from AcaChem Scientific (San Antonio, TX 78278. U.S.A.). 1-Beta-D-arabinofuranisyl was obtained from the EMD Millipore Corporation (Temecula, CA 92590, U.S.A.). Rupintrivir, carbodine, favipiravir, anisomycin, pirodavis, guanidine, cyanovirin, and nitazoxanide were provided by the Institute for Antiviral Research (IAR) chemical compound depository (Utah State University, UT 84321, U.S.A.). All compounds were dissolved in cell culture media (minimum essential medium with Earle's balanced salts and L-glutamine, MEM/EBSS) with gentamicin (50 µg/ml). Concentrations of 320, 32, 3.2, .32, .032, .0032, .00032, .000032 µg/ml were utilized for nitazoxanide, rupintrivir, carbodine, guanidine, favipiravir, azacytadine, 1-Beta-D-arabinofuranisyl. Concentrations of 100, 10, 1.0, .1, .01, .001, .0001, .00001 µg/ml were utilized for anisomycin, cyanovirin and pirodavis.

2.13 In Vitro Antiviral Studies Against Murine Norovirus

The antiviral activity of each drug or drug combination was determined in RAW 264.7 cells. Effective antiviral concentrations (EC50 values) were computed based on the inhibition of virus-induced cytopathic effects (CPE) coupled with a neutral red dye uptake method. Ninety-six-well plates were seeded with approximately 8×10^4 RAW 264.7 cells/well. Following overnight incubation, cells were infected with approximately 50 times the 50 % cell culture infectious dose (CCID50) of virus. Microtiter plates were visually examined 4 days post infection (dpi) and then treated for 2 h at 37 °C with neutral red (0.011 %) to quantify CPE. Excess dye was rinsed from cells with phosphate-buffered saline. The absorbed dye was eluted

by addition of 0.1 ml of 50 % Sorensen's citrate buffer/50 % ethanol to each well. Optical density (OD) measurements were taken using a computerized microplate reader (SPECTRAmax® Plus384, Molecular Devices Corporation, Sunnyvale, CA) with absorbance measurements at 560 nm. Optical density readings were converted to percent of uninfected control using an Excel spreadsheet by plotting CPE values versus \log_{10} of drug concentration.

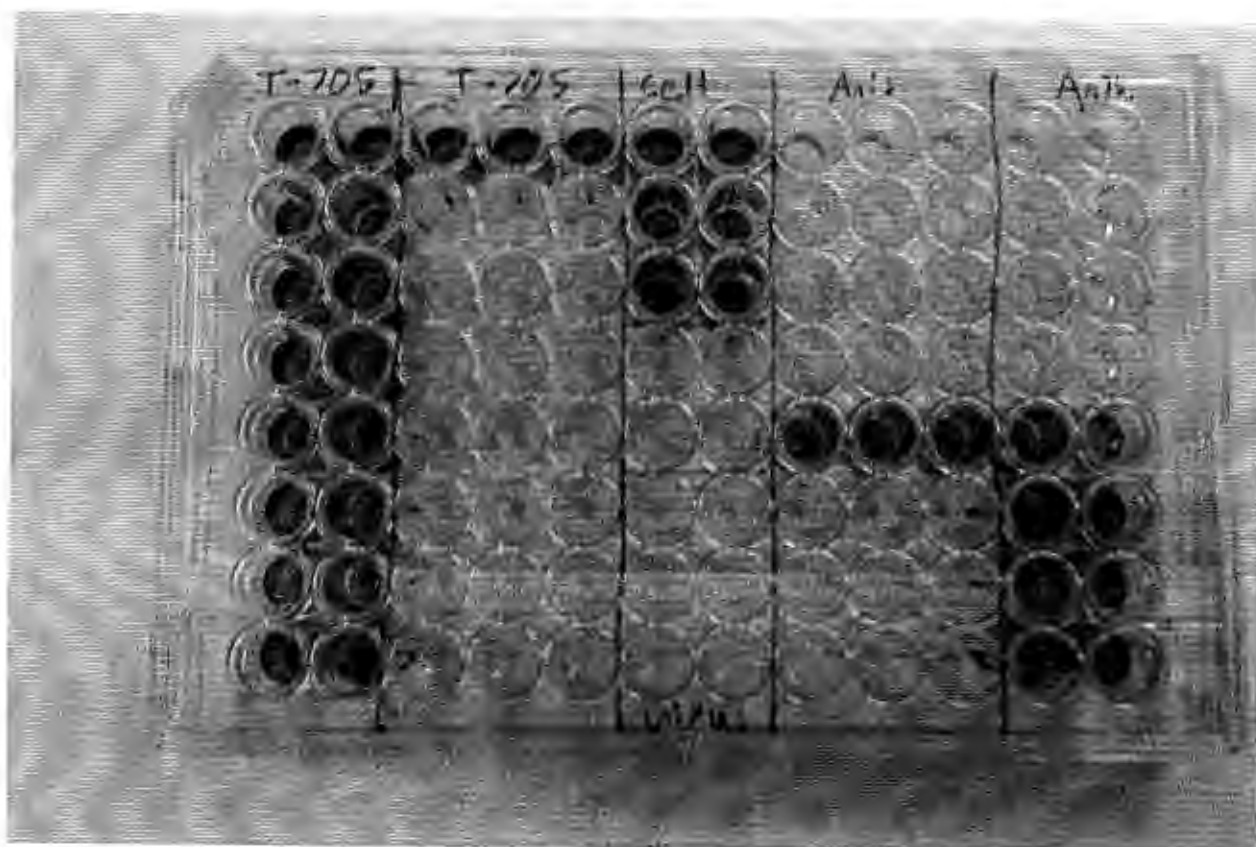


Figure 1: Standard plate format for antiviral evaluation. Maximal drug concentration is placed in the top well and a ten-fold dilution series is followed to the bottom well. The center four wells are a killed control. Center bottom six wells are a viral control. Center top six wells are a cell control.

2.14 Statistics

All confidence intervals were calculated using the confidence function in Microsoft Excel for Mac (version 16.11.1). Graphs and tables were also generated with Microsoft Excel for Mac

(version 16.11.1). P values were calculated using an unpaired student's t test from the Analysis ToolPak of Microsoft Excel for Mac (version 16.11.1). Variances were assumed unequal, a more conservative estimate than the assumption of equal variance. All P values were calculated using a two-tailed t test, which evaluates whether samples are different. If samples are found to be different, this test also confirms whether they are greater or lesser.

3 Results:

3.1 Initial 5 Passages of Influenza H3N2

Figure 2 shows the quantity of virus recovered from each subsequent mouse passage. Mouse to mouse passaging within AG-129 mice resulted in increase of influenza A/Hong Kong/4801/2014 H3N2 virus CCID50 over the first two to three passages, with mouse passage 1 (MP1) with a CCID50 of $10^{4.28}$ (95% confidence interval: $10^{3.5} - 10^{5.0}$) increasing to the viral titer of MP3, with a CCID50 of $10^{6.22}$ (95% confidence interval: $10^{5.5} - 10^{6.9}$). Following this viral concentration remained relatively constant through MP5 (Figure 2).

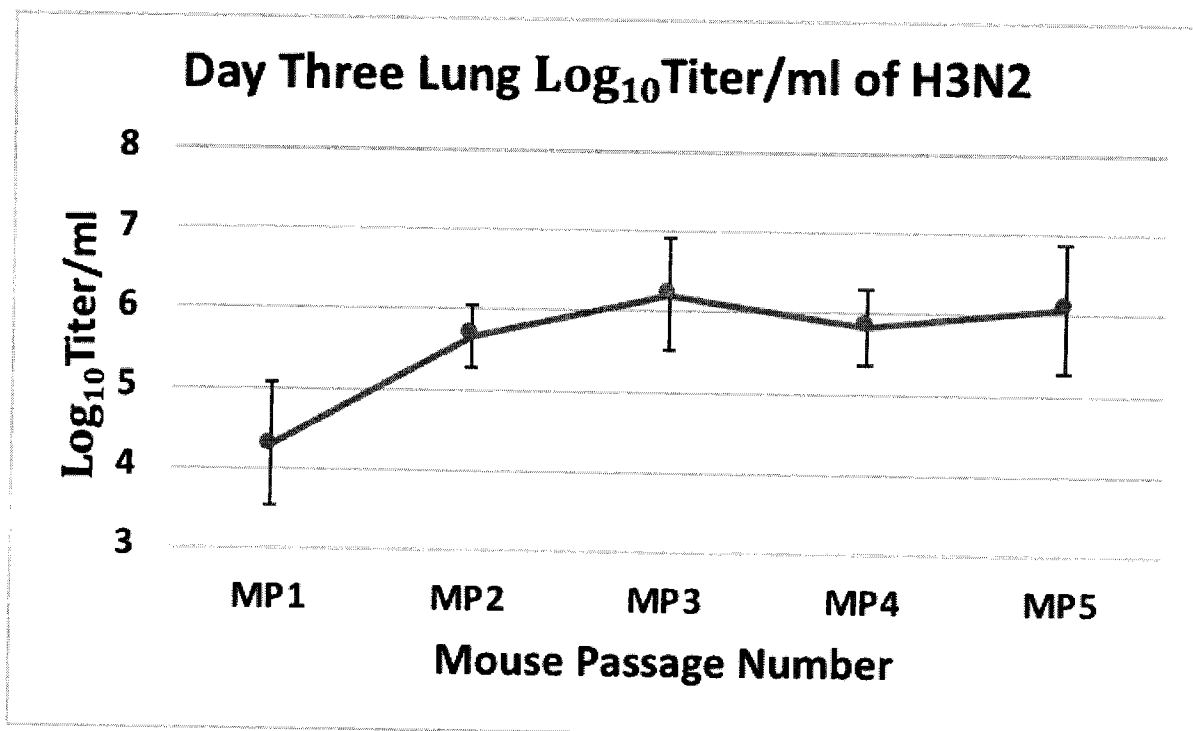


Figure 2: The x axis represents the Log₁₀(Titer)/ml or the Log₁₀(CCID₅₀)/ml. Mouse passage is represented with MP#. MP1 being mouse passage 1. All mouse passages were aided by mannan given concurrently with infection and intranasal rinses on day 1 and 2.

3.2 Evaluation of Disease Markers in MP4 Influenza H3N2

Intranasal inoculation with MP4 results both in weight loss, and in mortality in AG-129 mice. The surviving mouse dropped to a minimum weight of 18 grams, a 29.1% weight loss from its weight on the day of infection. A 33.8% weight loss from its maximal weight, which it reached one day post infection.

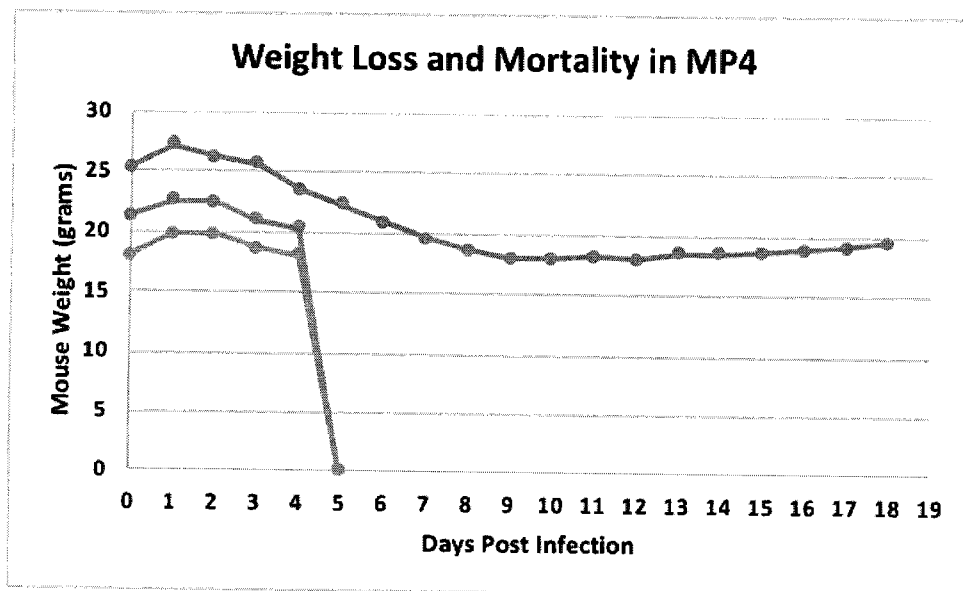


Figure 3: Evaluation of mortality and weight loss in AG-129 mice, inoculated intranasally with MP4 and mannan. Mice were given intranasal rinses on day 1 and day 2.

3.3 Influenza H3N2 Passages Without Mannan and Rinses

All three groups showed a reduction in viral concentration from their original passages to later passages (Figure 4 - 6). The two passages did not show a statistical difference between the original mouse passage (MP3 or MP5) and the two non-original stock passages. Figure 4 shows that mice inoculated with MP3, without intranasal rinses and mannan, had the greatest initial reduction in viral concentration, with a CCID50 of $10^{2.39}$ (95% confidence interval: $10^{2.28} - 10^{2.5}$). Figure five shows that mice inoculated with MP5 under the same conditions of MP3 performed better, with a recovered homogenate with a CCID50 of $10^{3.28}$ (95% confidence interval: $10^{2.99} - 10^{3.56}$). The highest CCID50 came from mice inoculated with MP5, with mannan but no rinses (figure 6). This passage had a CCID50 of $10^{4.8}$ (95% confidence interval: $10^{4.34} - 10^{5.33}$).

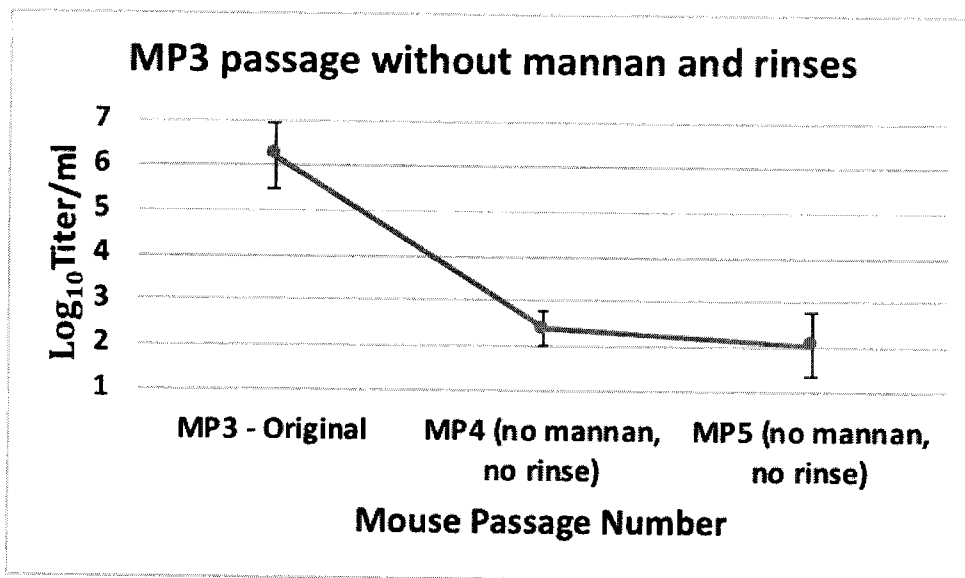


Figure 4: Mouse passage three (MP3), demarcated 'MP3 – Original' was passaged twice in the absence of mannan and intranasal rinses forming MP4 (no mannan, no rinse) and MP5 (no mannan, no rinse).

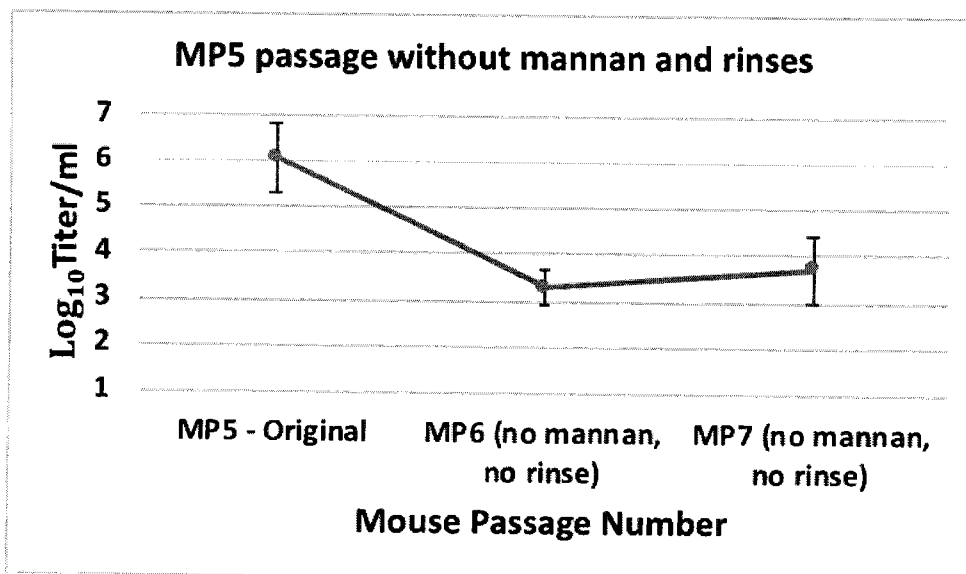


Figure 5: Mouse passage three (MP5), demarcated 'MP5 – Original' was passaged twice in the absence of mannan and intranasal rinses forming MP6 (no mannan, no rinse) and MP7 (no mannan, no rinse).

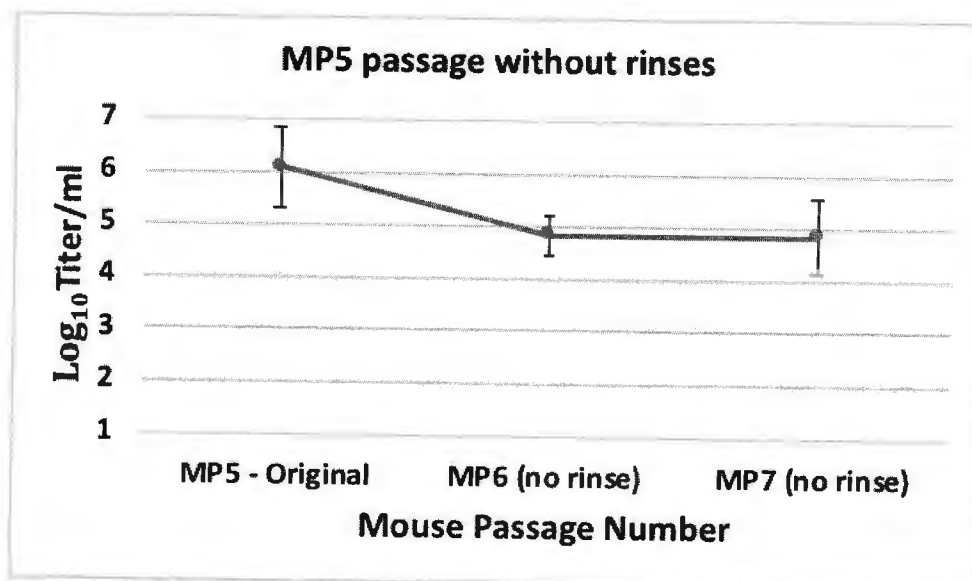


Figure 6: Mouse passage three (MP5), demarcated 'MP5 – Original' was passaged twice in the absence of intranasal rinses forming MP6 (no rinse) and MP7 (no rinse).

3.4 In Vitro Screening of Compounds Against Murine Norovirus

Of all compounds tested, only rupintrivir, carbodine, favipiravir and anisomycin displayed antiviral activity. Figure 7 shows the selectivity index of these four antivirals, as well as their cytotoxic concentration 50% (CC50) and their effective concentration 50% (EC50).

Compound	CC50	EC50	SI
Rupintrivir	7 ug/ml	7 ug/ml	1
Carbodine	> 320 ug/ml	10 ug/ml	> 32
Favipiravir	> 320 ug/ml	100 ug/ml	> 3.2
Anisomycin	9.5 ng/ml	3.1 ng/ml	3.1

Figure 7: The cytotoxic concentration 50% (CC50) is a measure of at what concentration the drug killed 50% of the cells. Effective concentration 50% (EC50) is a measure of at what concentration the compound saved 50% of the cells. Selectivity index is the CC50 divided by EC50. It therefore represents how selective a compound is for the viral target versus the host cell.

4 Discussion:

4.1 Influenza H3N2 Murine Model:

Figure 2 showed an initial increase in viral CCID50 after serial passaging. The rise in viral quantity from MP1 to MP2, an increase in over a order of magnitude, suggests de novo replication of influenza H3N2 in AG-129 mice. A result which is supported by figure 3, which shows mortality and weight loss resulting from infection with MP4. In addition, figure 3 also shows that when aided with mannan and by day 1 and 2 intranasal rinses, the virus is capable of showing disease. Before this can be concluded definitively however, multiple steps must be taken. The virus would need to be plaque purified, and confirmed, by PCR or other methods, to be Influenza A Hong Kong/4801/2014 H3N2 virus. Furthermore, a larger study giving more statistical certainty to the frequency of mortality and disease signs would need to be performed. Finally, a compound with known efficacy against influenza H3N2 would ideally be tested in the model. Confirming whether it is possible to protect against mortality by use of a therapeutic in the model and showing whether there is crossover between the model and instances of human infection.

Figures 4 and 5 shows that passaging of H3N2 without mannan and intranasal rinses results in a decrease concentration of virus. The reduction in MP3 to MP4 (no mannan, no rinses), was greater than the reduction in MP5 to MP6 (no mannan, no rinse) ($p = .0109$). Showing MP5 has developed either increased ability to survive or increased ability to replicate relative to MP3. Figure 6 when compared with figure 5 shows that MP5 had either increased replication or survival when assisted by mannan than when not assisted by mannan ($p = .013$).

De novo replication of MP5 or MP3 without mannan and intranasal rinses is likely, as neither mannan or intranasal rinses increase the rate of viral replication. If, however, the virus is

replicating deep within the lungs, intranasal rinsing may be necessary for the virus to reach the cells within which it undergoes replication. Further passaging will discern whether virus levels fall, remain constant or rise. Either a rise or constant level of virus suggest de novo replication. This is because each subsequent passage involves homogenization of virus in 1 ml of MEM and then infection with 90 μ l of this stock. In other words, the virus undergoes a 1:11 dilution each passage. This reduction may be more than a 1:11 dilution, as all virus injected intranasally may not reach the lungs. Considering this, consistently retrieving constant levels of virus from one passage to the next is a sign of de novo replication.

4.2 Antiviral Screening Against Murine Norovirus:

While rupintrivir displayed activity, its selectivity index is too low for therapeutic use. Anisomycin also displays a small selectivity index. In addition to this, anisomycin has a low CC₅₀ at 9.5 ng/ml, meaning the compound displays high toxicity. The lack of an upper bound on the CC₅₀ values of carbodine and favipiravir results because no toxicity was seen at the concentrations used on the plate. A definitive selectivity index for these compounds would require an additional screen, which utilizes higher concentrations of the compound than 320 μ g/ml.

Of the efficacious compounds, carbodine and favipiravir both show therapeutic potential (Figure 7). Carbodine with a selectivity index of at least 32, showed the greatest potential. Possible future steps include the evaluation of carbodine in the AG-129 lethal model for murine norovirus. While the compound has potential, carbodine is costly, at over 500 dollars for 5 mg. Compounds with similar structures such as gentamycin, 5-azacytadine and 1-beta-D-Arabinofuranisyl, could be tested against murine norovirus and may offer cheaper alternatives.

Finally, compounds found efficacious against murine norovirus could be screened in vitro against human norovirus. This could provide an indication as to whether murine norovirus infection resembles human norovirus infection closely enough to act as a valid model for therapeutic evaluation.

5 Conclusion:

5.1 Influenza H3N2 Murine Model

Levels of Influenza A Hong Kong/4801/2014 H3N2 virus following mouse passage one showed an increase, suggesting adaption of virus and de novo replication (figure 2). Mouse passage four was capable of mortality and weight loss (figure 3), when virus was given concurrently with mannan and intranasal rinses were performed day one and two. This further suggests de novo replication. It also indicates potential as a therapeutic model. PCR verification of viral strain has not yet been performed, so it is possible a contaminant has caused the increase in virulence. Passage of MP3 and MP5 without mannan and intranasal rinses resulted in a net reduction of virus (Figures 4 – 6) after initial passaging. The subsequent passage did not cause a statistically significant change in the quantity of the virus.

If stable replication occurs without mannan and intranasal rinses in future passages, then further projects may endeavor to adapt this future passage to replication within BALB/c mice. These mice provide a less expensive alternative to AG-129 mice and may prove a better model given that they possess normal interferon responses.

5.2 Antiviral Screening Against Murine Norovirus

Of the compounds tested, rupintrivir, anisomycin, favipiravir, and carbodine showed efficacy (figure 7). Of these efficacious compounds favipiravir and carbodine possess the highest selectivity indices, at >3.2 and >32 respectively. Favipiravir and carbodine selectivity indices lack an upper bound as they displayed no toxicity at the maximal concentration tested (320 µg/ml).

Possible future directions include; evaluation of compounds in vitro in the human norovirus model, identifying the upper bounds of the selectivity indices, in vivo evaluation against murine norovirus, and screening of compounds in the in vitro model of human norovirus.

References

- [1] "Frequently Asked Flu Questions 2017-2018 Influenza Season." Centers for Disease Control and Prevention, Centers for Disease Control, 30 Mar. 2018, www.cdc.gov/flu/about/season/flu-season-2017-2018.htm.
- [2] "Weekly U.S. Influenza Surveillance Report." Centers for Disease Control and Prevention, Centers for Disease Control, 13 Apr. 2018, www.cdc.gov/flu/weekly/index.htm.
- [3] "Norovirus Worldwide." Centers for Disease Control and Prevention, Centers for Disease Control, 24 June 2016, www.cdc.gov/norovirus/worldwide.html.
- [4] Bouvier, Nicole M., and Peter Palese. "THE BIOLOGY OF INFLUENZA VIRUSES." *Vaccine* 26.Suppl 4 (2008): D49–D53. Print.
- [5] "Influenza (Seasonal)." World Health Organization, World Health Organization, 13 Mar. 2018, www.who.int/mediacentre/factsheets/fs211/en/.
- [6] Molinari, Noelle-Angelique M., et al. "The Annual Impact of Seasonal Influenza in the US: Measuring Disease Burden and Costs." *Vaccine*, vol. 25, no. 27, 2007, pp. 5086–5096., doi:10.1016/j.vaccine.2007.03.046.
- [5] "Influenza (Seasonal)." World Health Organization, World Health Organization, 13 Mar. 2018, www.who.int/mediacentre/factsheets/fs211/en/.
- [7] "Types of Influenza Viruses." Centers for Disease Control and Prevention, Centers for Disease Control, 27 Sept. 2017, www.cdc.gov/flu/about/viruses/types.htm.
- [8] Bartsch, Sarah M., et al. "Global Economic Burden of Norovirus Gastroenteritis." *Plos One*, vol. 11, no. 4, 2016, doi:10.1371/journal.pone.0151219
- [9] . Ha, Sangdo, et al. "Infection Models of Human Norovirus: Challenges and Recent Progress." *Archives of Virology*, vol. 161, no. 4, 2016, pp. 779–788., doi:10.1007/s00705-016-2748-4.
- [10] Thorne, L. G., and I. G. Goodfellow. "Norovirus Gene Expression and Replication." *Journal of General Virology*, vol. 95, no. Pt_2, 2013, pp. 278–291., doi:10.1099/vir.0.059634-0.

[11] Mcfadden, Nora, et al. "Norovirus Regulation of the Innate Immune Response and Apoptosis Occurs via the Product of the Alternative Open Reading Frame 4." *PLoS Pathogens*, vol. 7, no. 12, 2011, doi:10.1371/journal.ppat.1002413.

[12] Rocha-Pereira, J., et al. "The Viral Polymerase Inhibitor 2'-C-Methylcytidine Inhibits Norwalk Virus Replication and Protects against Norovirus-Induced Diarrhea and Mortality in a Mouse Model." *Journal of Virology*, vol. 87, no. 21, 2013, pp. 11798–11805., doi:10.1128/jvi.02064-13.

[13] Taylor, R. M. "EXPERIMENTAL INFECTION WITH INFLUENZA A VIRUS IN MICE." *JEM*, Rockefeller University Press, 1 Jan. 1941, jem.rupress.org/content/73/1/43.

[14] Guo, Jinlei, et al. "Limited Effect of Recombinant Human Mannose-Binding Lectin on the Infection of Novel Influenza A (H7N9) Virus In Vitro." *Biochemical and Biophysical Research Communications*, vol. 458, no. 1, 2015, pp. 77–81., doi:10.1016/j.bbrc.2015.01.070.

[15] Kase, et al. "Human Mannan-Binding Lectin Inhibits the Infection of Influenza A Virus without Complement." *Immunology*, vol. 97, no. 3, 1999, pp. 385–392., doi:10.1046/j.1365-2567.1999.00781.x.

[16] Smee, Donald F, et al. "Treatment of Mannan-Enhanced Influenza B Virus Infections in Mice with Oseltamivir, Ribavirin and Viramidine." *Antiviral Chemistry and Chemotherapy*, vol. 15, no. 5, 2004, pp. 261–268., doi:10.1177/095632020401500505.

Author Autobiography:

I was born and raised in a small town in Stansbury Park, with three brothers, no sisters, and two very patient parents. I started attending Utah State University in the fall 2013. In Spring of 2015, I started working at the Institute for Antiviral Research as laboratory technician. Since then I have done research projects working with Dr. Tarbet, Dr. Welker, and Dr. Julander. I will be graduating with dual degrees in Biology and Animal Dairy and Veterinary Science with minors in Chemistry and Computer Science at the end of spring 2018 semester. Following graduation, I will be attending Oregon Health and Science University, where I have been accepted into the Program for Molecular and Cellular Biosciences for completion of PhD.

Reflective Writing

During my years at Utah State University I dual majored in animal dairy and veterinary science and biology as well as two minors, one in chemistry and one in computer science. In addition to my academic work, I have worked three years for the Institute for Antiviral Research here at Utah State University for the College of Agriculture and Applied Sciences. When building a capstone experience, I wished to be able to expand both my academic knowledge of majors and my knowledge of the field of virology.

My time here at USU provided me with an excellent understanding of in vivo antiviral evaluation but I had done little work performing antiviral screens inside in vitro model systems. In addition to a desire to explore different aspects of antiviral investigation, I also wanted the opportunity to work independently, albeit supervised by a principle investigator, on a research project of my own.

While these interests are in part meant to expound upon what I have enjoyed at Utah State University, they are also intended to help me in my future pursuits. I intend to get a PhD in Virology, which requires independent experience and expertise on my resume.

To achieve this, I reached out to Dr. E. Bart Tarbet, a principle investigator at the Institute of Antiviral Research who works on a variety of projects in virology, including vaccine evaluation, antiviral testing, and murine model development. We discussed my interests, and the reasons for them, with Dr. Tarbet and we decided on three projects that I could work on for my thesis. One project, was the adaption of influenza A subtype H3N2 into in murine model in AG-129 mice (immune incompetent mice). These mice were chosen as previous attempts to adapt the virus in immune competent mice, the BALB/c strain, had failed. In addition to the

adaption of influenza A virus, subtype H3N2, I also worked two other projects namely; the adaption of human rhinovirus and respiratory syncytial virus to an AG-129 host. We were unable to get either of these viruses to grow within mice, which supplied additional time in my schedule for another project.

After conversing with Dr. Tarbet once again, it was decided that in addition to mouse passaging for the adaption of influenza virus A subtype H3N2 I would also screen compounds in vitro against murine norovirus. Murine norovirus is a model system which acts as an analog of human noroviruses. This model is often necessitated by the difficulty of growing human noroviruses both in vitro and in vivo in any animal but the natural human host.

Both these opportunities brought new experiences with them. Initially my time was split between the laboratory and the scientific literature. When in the laboratory, I reached out to other members of the institute for antiviral research, asking for their instruction on how to perform techniques and assays new to me. This broadened my skillset, increasing my future marketability within the field. When outside the laboratory, I looked over articles describing the mechanism of action of various antiviral compounds as well as the life cycle of human and murine noroviruses. There would be no use in finding an antiviral compound which had already been discovered, so knowing which compounds had been found to have efficacy was valuable information. In addition to this knowledge, by learning the mechanism of action of an antiviral compounds, and relying on others researcher's descriptions of murine noroviruses lifecycle I could predict which compounds might have efficacy in vitro.

This process proved itself invaluable to me as it allowed me to acclimatize myself to research articles and other technically written documents. In addition, I was permitted to

develop hypotheses, develop a potential design for a study, request authorization from Dr. Tarbet, and then evaluate the hypothesis within the laboratory. I repeated this process until I had uncovered four potential antiviral compounds, which I will be investigating further over this summer.

In addition to the antiviral work, my studies on viral adaption for murine model development also required me to adjust my methods as I acquired new information. Dr. Tarbet and I's first hypothesis, namely that adaption within AG-129 mice would allow sufficient respite from immunity for the virus to begin replicating and evolving to life in a murine host was not correct. Initially passaging of all three viruses was unsuccessful. This provided me with another opportunity to peruse through scientific journals, this time in search of techniques which can aid in viral adaption within the host. Thanks to this search, and to advice from discussions with members of my laboratory, mannan and intranasal saline treatments were both identified as a potential aide for adaption. Mannan acted as a pathogen recognition receptor inhibitor, specifically by competing for the potential binding sites of the mannan-binding lectins with influenza A subtype H3N2, helping to spare them from contact with these receptors. Intranasal rinsing was utilized to wash the virus deeper into the lungs, and expose it to new tissue and cells for growth.

These adaption techniques were effective for influenza A subtype H3N2. Having had success with adaption and antiviral screening the next step for my project was to make a written product, my bachelor's thesis, and two products which would aide in oral presentation of the material a poster and a powerpoint presentation. This provided an excellent opportunity for me to become more familiar with statistics, a branch of mathematics often utilized in

scientific fields. In addition to this it provided an opportunity for me to share my work, both with professors, during the defense of my thesis, and with other students, when I presented my work at the biology undergraduate research symposium. At this symposium, I was rewarded a prize for best presentation, showing that this project has provided me with an opportunity to work at both the practice of science and on the art of explaining science clearly and enthusiastically to others.

In summation, this project embodied the ideal honors capstone project for me because it allowed me to: investigate more deeply into virology, spend more time reading in scientific literature, explore the scientific methods, to test hypotheses, perform independent research, and to explain my new understanding to individuals within my local community.